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A Retrospective Study on Insulin Resistance in Adults with Autoimmune Thyroiditis in Turkish Population: a Clinical and molecular Docking Study

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ABSTRACT

Purpose: This study retrospectively analyzes data to assess the prevalence and severity of insulin resistance in adults with autoimmune thyroiditis, including Hashimoto's thyroiditis. Additionally, 14 compounds from the methanolic extract of G. aparine, traditionally used for hypothyroidism, were docked into the Thyroid Hormone Receptor binding site. Luteolin, Quercetin, and Esculetin were predicted to bind successfully to the receptor.

Methods: Our approach involves a comprehensive review of medical records to evaluate insulin levels, HbA1c values, and other health markers in patients with autoimmune thyroiditis. Molecular docking simulations were performed using the Schrödinger suite, and data analysis was conducted with SPSS version 26. Normality of distribution was verified using the Shapiro-Wilks test and Box Plot diagrams, and correlations between variables were determined using Pearson or Spearman's correlation analysis.

Results: Our analysis showed a significant positive correlation between higher Insulin Resistance (HOMA-IR) scores and increased blood glucose and insulin levels ($p<0.01$). There was also a moderate positive correlation between HOMA-IR scores and both HbA1c and HbA1c (IFCC) levels. In molecular docking simulations, Luteolin and Quercetin provided greater stabilization than Esculetin due to bonds at both ends and larger molecular size, resulting in higher binding scores.

Conclusion: This study highlights the significant impact of insulin resistance in individuals with autoimmune thyroiditis, offering insights for improved diagnosis and treatment. Additionally, molecular docking simulations show that Luteolin, Quercetin, and Esculetin from the traditional medicinal plant G. aparine, used for hypothyroidism, successfully bind to the Thyroid Hormone Receptor. Understanding these associations can enhance clinical strategies and guide further research to improve treatment outcomes.

Keywords: Insulin resistance, Autoimmune thyroiditis, Hashimoto's thyroiditis, Glucose metabolism, HbA1c

ÖZET

Amaç: Bu çalışma, Hashimoto tiroiditi dahil olmak üzere otoimmün tiroiditli yetişkinlerde insülin direncinin yaygınlığını ve şiddetini değerlendirmek için verileri retrospektif olarak analiz etmeyi amaçlamıştır. Ayrıca, geleneksel olarak hipotiroidizm için kullanılan G. aparine'nin metanolik ekstraktından 14 bileşik, Tiroid Hormonu Reseptörü bağlanma bölgesine yerleştirilmiştir. Luteolin, Kuersetin ve Eskuletin'in reseptöre başarıyla bağlanacağı öngörülmüştür.

Yöntem: Yaklaşımımız, otoimmün tiroiditli hastalarda insülin seviyeleri, HbA1c değerleri ve diğer sağlık belirteçlerini değerlendirmek için tıbbi kayıtların kapsamlı bir şekilde incelenmesini içermektedir. Moleküler yerleştirme simülasyonları Schrödinger paketi kullanılarak gerçekleştirilmiş ve veri analizi SPSS versiyon 26 ile yapılmıştır. Dağılımın normalliği Shapiro-Wilks testi ve Box Plot diyagramları kullanılarak doğrulanmış, değişkenler arasındaki korelasyonlar Pearson veya Spearman korelasyon analizi ile belirlenmiştir.

Bulgular: Analizimiz, daha yüksek İnsülin Direnci (HOMA-IR) skorları ile artan kan şekeri ve insülin seviyeleri arasında anlamlı bir pozitif korelasyon göstermiştir ($p<0.01$). Ayrıca, HOMA-IR skorları ile hem HbA1c hem de HbA1c (IFCC) seviyeleri arasında orta derecede pozitif bir korelasyon bulunmuştur. Moleküler yerleştirme simülasyonlarında, Luteolin ve Kuersetin, her iki uçtaki bağlar ve daha büyük moleküler boyutları nedeniyle Eskuletin'den daha fazla stabilizasyon sağlamış ve bu da daha yüksek bağlanma skorları ile sonuçlanmıştır.

Sonuç: Bu çalışma, otoimmün tiroiditli bireylerde insülin direncinin önemli etkisini vurgulayarak, teşhis ve tedavi için iyileştirilmiş yaklaşımlar sunmaktadır. Ayrıca, geleneksel olarak hipotiroidizm için kullanılan tıbbi bitki G. aparine'den elde edilen Luteolin, Kuersetin ve Eskuletin'in Tiroid Hormonu Reseptörüne başarıyla bağlandığını gösteren moleküler yerleştirme simülasyonları yapılmıştır. Bu ilişkilerin anlaşılması, klinik stratejileri geliştirebilir ve tedavi sonuçlarını iyileştirmek için daha fazla araştırmaya yön verebilir.

Anahtar Kelimeler: İnsülin direnci, Otoimmün tiroidit, Hashimoto tiroiditi, Glukoz metabolizması, HbA1c

Hashimoto's thyroiditis, also known as chronic lymphocytic or autoimmune thyroiditis, is a long-term autoimmune condition characterized by the immune system producing thyroid peroxidase (TPO) and/or thyroglobulin (Tg) antibodies that attack the thyroid gland (1, 2). This attack leads to inflammation and a slow decline in thyroid function, often resulting in hypothyroidism, where the thyroid produces insufficient hormones (3). Hypothyroidism can decrease glucose absorption, causing sugar accumulation and increased insulin production, eventually leading to insulin resistance and potentially diabetes (4-7). Comprehensive management of Hashimoto's thyroiditis should address both thyroid function and its metabolic consequences (8). It is imperative to develop a novel therapy with minimal or no side effects that can enhance thyroid function. Plant-based medicines are often considered as the potential options that provide valuable therapy for various diseases with negligible or no side effects (9).

Plants have been a source of natural medicine since ancient times, with 25–48% of drugs today originating from plants or their synthetic derivatives (10). *Galium aparine* L. (GA), also known as Cleavers or "Yogurt herb" due to its use in cheese manufacturing, is common in Anatolia and traditionally used for hypothyroidism. Fourteen compounds were identified in the methanolic extract of *G. aparine* (11). This study also aims to elucidate the molecular mechanism of action using in silico methods, specifically molecular docking simulations.

In this study, our objective was to assess the impact of insulin resistance in adult populations with proven autoimmune thyroiditis. We conducted a retrospective screening using routine blood test results and employed statistical methods to evaluate insulin resistance levels in individuals with autoimmune thyroiditis. By utilizing retrospective screening methods, we aimed to provide valuable insights into the potential relationship between autoimmune thyroiditis and insulin resistance.

To support these findings; we also aimed to perform the mechanism of action at the molecular level using established in silico methods, specifically molecular docking simulations. Our findings will contribute to a better understanding of the metabolic implications of autoimmune thyroiditis and shed light on the importance of assessing insulin resistance in this population.

Material and Methods

Experimental Design

The ethical oversight for this investigation was provided by the Ethics Committee of Biruni University, which granted approval under the protocol number 2022/75-12. This research involved a cohort of 34 participants from whom various biomarkers were measured, including hemoglobin, insulin, HbA1c (hemoglobin A1c), anti-TPO (thyroid peroxidase antibodies), anti-Thyroglobulin, ferritin, vitamin B12, and vitamin D levels.

Molecular Docking

Molecular docking simulations were performed using Schrödinger suite (12). Crystal structure of Thyroid Hormone Receptor with 2.20 Å resolution was obtained from protein databank (PDB ID: 3GWS). The raw 3D structure was initially prepared by adding hydrogen atoms, correcting bond orders and minimizing the system after optimizing step. All compounds to be docked were also prepared using LigPrep (13) module implemented in Schrödinger suite. In order to cover large chemical space, all possible conformations and tautomers were also considered and ionization states at pH 7.0±2 were generated too. Glide's standard precision (SP) method was conducted for docking of all structure into the receptor.

Statistical Analysis

The study used descriptive and inferential statistical methods to analyze biomarkers, summarizing continuous and qualitative variables. SPSS version 26 was used for analysis, with normality assessed using Shapiro-Wilks test and Box Plot graphs, and correlations were evaluated using Pearson or Spearman's analysis ($p < 0.05$). The study excluded participants with thyroid medication, antioxidant supplementation, insulin resistance, autoimmune diseases, pregnancy, thyroid surgery, or thyroid cancer, and used anonymous results for interpretation.

Results

The study explores the link between autoimmune thyroiditis and insulin resistance, using biomarkers and blood test results. It aims to understand the metabolic implications of autoimmune thyroiditis and improve management and treatment strategies for individuals with the condition.

Data from 34 participants at Dr. Savaş Gur's outpatient clinic were analyzed for gender representation. Among them, 14.7% (n=5) were males and 85.3% (n=29) were females, reflecting the higher prevalence of autoimmune thyroiditis in females (Figure 1A). The graphical representation of gender distribution offers a visual overview of the study population, aiding in the interpretation of findings.

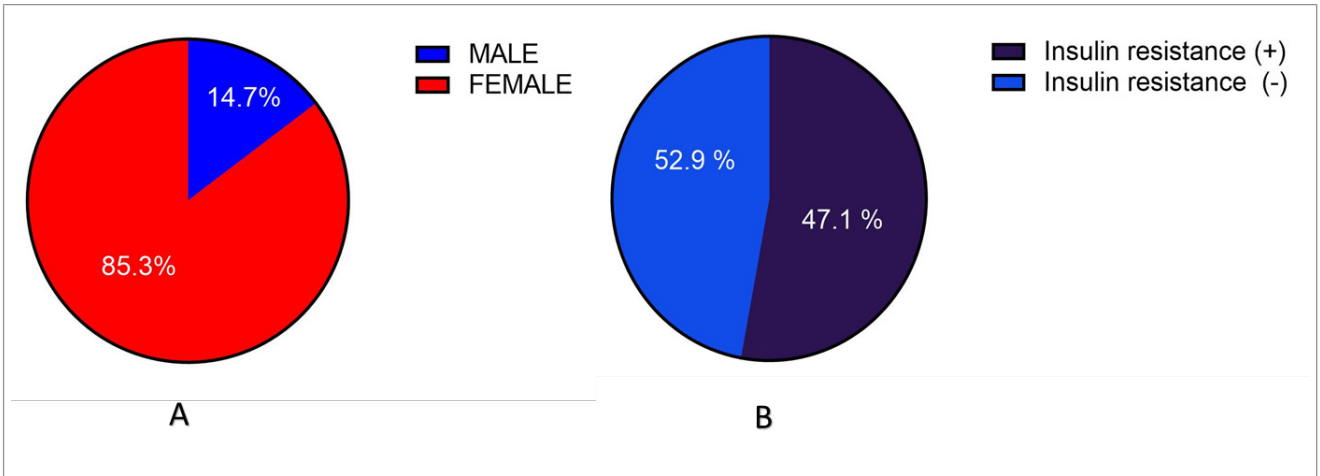

Figure 1: A) The distribution of genders. This figure illustrates the distribution of genders within our study population.
B) Distribution of Insulin Resistance. This figure displays the distribution of insulin resistance among the study participants.

Table 1 provides a comprehensive overview of the clinical measurements obtained from the study participants. The table includes various hematological, metabolic, and immunological parameters along with their corresponding mean and standard deviation values. The average hemoglobin level was 13.25±1.55, insulin resistance (HOMA-IR) was 3.40±2.44, blood sugar was 101.82±38.96, insulin was 13.28±7.57, HbA1c (both in percentage and IFCC units) were 5.58±1.11 and 37.62±12.18 respectively, anti-thyroid peroxidase (TPO) antibodies were 188.98±153.31, anti-thyroglobulin antibodies were 310.89±621.58, ferritin was 67.41±83.14, vitamin B12 was 445.94±193.04, and vitamin D was 24.18±12.68. These descriptive statistics provide valuable insights into the central tendencies and variability of the measured parameters within the study population, serving as a basis for further analysis and interpretation of the data.

Table 1: Distribution of Clinical Measurements

	Mean±Sd	Median (Min-Max)
Hemoglobin	13,25±1,55	13,2 (10,3-17,5)
Insulin resistance HOMA-IR	3,40±2,44	2,7 (0,8-12,4)
Blood sugar	101,82±38,96	94 (71-261)
Insulin	13,28±7,57	10,7 (3,8-32,4)
HbA1c	5,58±1,11	5,3 (4,6-10,8)
HbA1c IFCC	37,62±12,18	34,5 (27-95)
Anti TPO	188,98±153,31	150,9 (8,9-540)
Anti Triglobulin	310,89±621,58	144,5 (15,9-3600)
Ferritin	67,41±83,14	46 (7-460)
Vitamin B12	445,94±193,04	424 (161-969)
Vitamin D	24,18±12,68	21,5 (6-62)

r=Pearson's Correlation Test
***p<0,01*

The data shows that 52.9% of the study population had insulin resistance, while 46.1% did not. Figure 1B visually represents this prevalence. Understanding insulin resistance distribution is crucial for assessing its impact on study outcomes and implications for participant management and treatment.

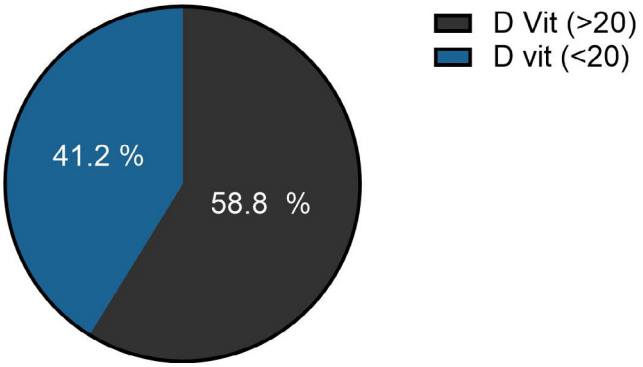

Figure 2: Distribution of Vitamin D. This figure depicts the distribution of vitamin D levels among the patients.

The data indicates that 58.8% of the patients had vitamin D levels greater than 20, while the remaining 41.2% had vitamin D levels less than or equal to 20. Figure 2 provides a visual representation of the proportion of patients with sufficient (above 20) and insufficient (20 or below) vitamin D levels within the studied population. Understanding the distribution of vitamin D levels is crucial for evaluating the prevalence of vitamin D deficiency and its potential impact on the patients' health and well-being.

Table 2 shows a significant positive correlation between higher HOMA-IR values and elevated blood glucose (p=0.556; p=0.001), insulin (p=0.829; p=0.001), HbA1c (p=0.699; p=0.001), and HbA1c (IFCC) levels (p=0.706; p=0.001). These findings suggest that higher HOMA-IR values are linked to increased blood glucose, insulin levels, and long-term glucose control, highlighting the importance of understanding insulin resistance to develop effective treatment strategies.

Table 2: Relationship between Insulin Resistance (HOMA-IR) and Clinical Measurements

	Insulin resistance (HOMA-IR)	
	r	p
Hemoglobin	0,212	***0,229***
Blood sugar	0,556	***0,001****
Insulin	0,829	***0,001****
HbA1c	0,699	***0,001****
HbA1c (IFCC)	0,706	***0,001****
Anti TPO	-0,043	***0,811***
Anti Triglobulin	0,243	***0,166***
Ferritin	-0,107	***0,546***
Vitamin B12	-0,051	***0,776***
Vitamin D	-0,085	***0,633***

Figure 3 shows no significant correlation between insulin resistance (HOMA-IR) and clinical indicators like hemoglobin, anti-TPO, anti-thyroglobulin, ferritin, vitamin B12, and vitamin D ($p > 0.05$). Figure 4 illustrates the relationships between vitamin D and hemoglobin, anti-thyroglobulin, and vitamin B12 levels. These findings suggest the complexity of insulin resistance's etiology and highlight the need for further research to explore additional factors.

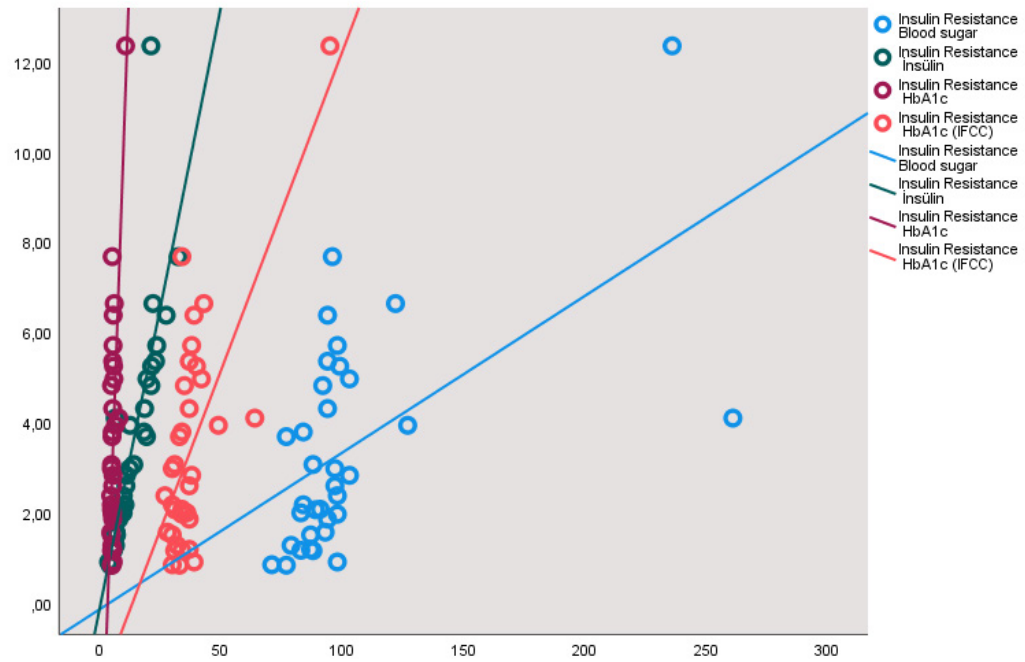

Figure 3: Relationship between Insulin Resistance (HOMA-IR) and blood glucose, HbA1c, and HbA1c (IFCC) measurements.

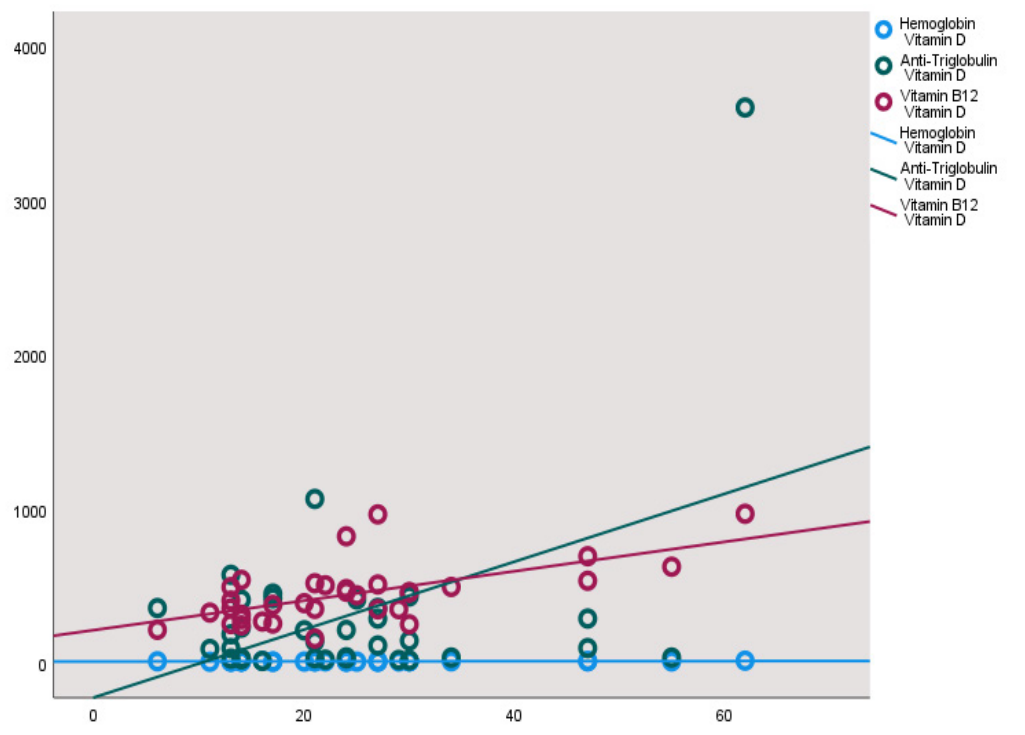

Figure 4: Relationship between Vitamin D and Hemoglobin, Anti Tri globulin and Vitamin B12 measurements

Table 3 shows Vitamin D levels have a weak positive correlation with hemoglobin (p=0.348; p<0.05) and Anti-Thyroglobulin (p=0.448; p<0.01), and a moderate positive correlation with Vitamin B12 (p=0.625; p<0.01). No significant relationships were found between Vitamin D and blood glucose, insulin, HbA1c, HbA1c (IFCC), Anti-TPO, and Ferritin (all p>0.05), highlighting Vitamin D's selective impact on certain clinical measures.

Table 3: The Relationship Between Vitamin D and Clinical Measurements

	Vitamin D	
	r	***p***
Hemoglobin	0,348	*0,044**
Blood sugar	-0,054	*0,762*
Insulin	-0,073	*0,683*
HbA1c	-0,074	*0,678*
HbA1c (IFCC)	-0,072	*0,684*
Anti TPO	0,148	*0,404*
Anti Triglobulin	0,448	*•0,008***
Ferritin	0,040	*0,821*
Vitamin B12	0,625	*0,001***

r=Pearson's Correlation Test
*r•=Spearman's Correlation Test **p<0,01*

In Table 4, Luteolin, Quercetin, and Esculetin show promising binding scores compared to the co-crystallized ligand. Figure 5 illustrates the binding interactions of all four compounds, including T3. A hydrogen bond interaction with Asparagine 331 is observed in all cases, suggesting its crucial role in ligand activity against the Thyroid Hormone Receptor.

Table 4: Docking scores of the native ligand T3 and the studied compounds.

Compound	Docking Score (kcal/mol)
3GWS-Native Ligand, T3	-10.30
Luteolin	-10.19
Quercetin	-9.36
Esculetin	-8.62
Coumarin	-7.61
Monotropein	-7.26
Normetanephrine	-6.77
Chlorogenic-acid	-6.28
p-Coumaric-acid	-6.19
Cinnamic-acid	-5.86
Pyridoxine	-5.72
Spectinomycin	-5.64
Pantothenic-acid	-3.62

The study underscores the need for further research on Vitamin D's health effects, highlighting its limited influence on other parameters in this particular group.

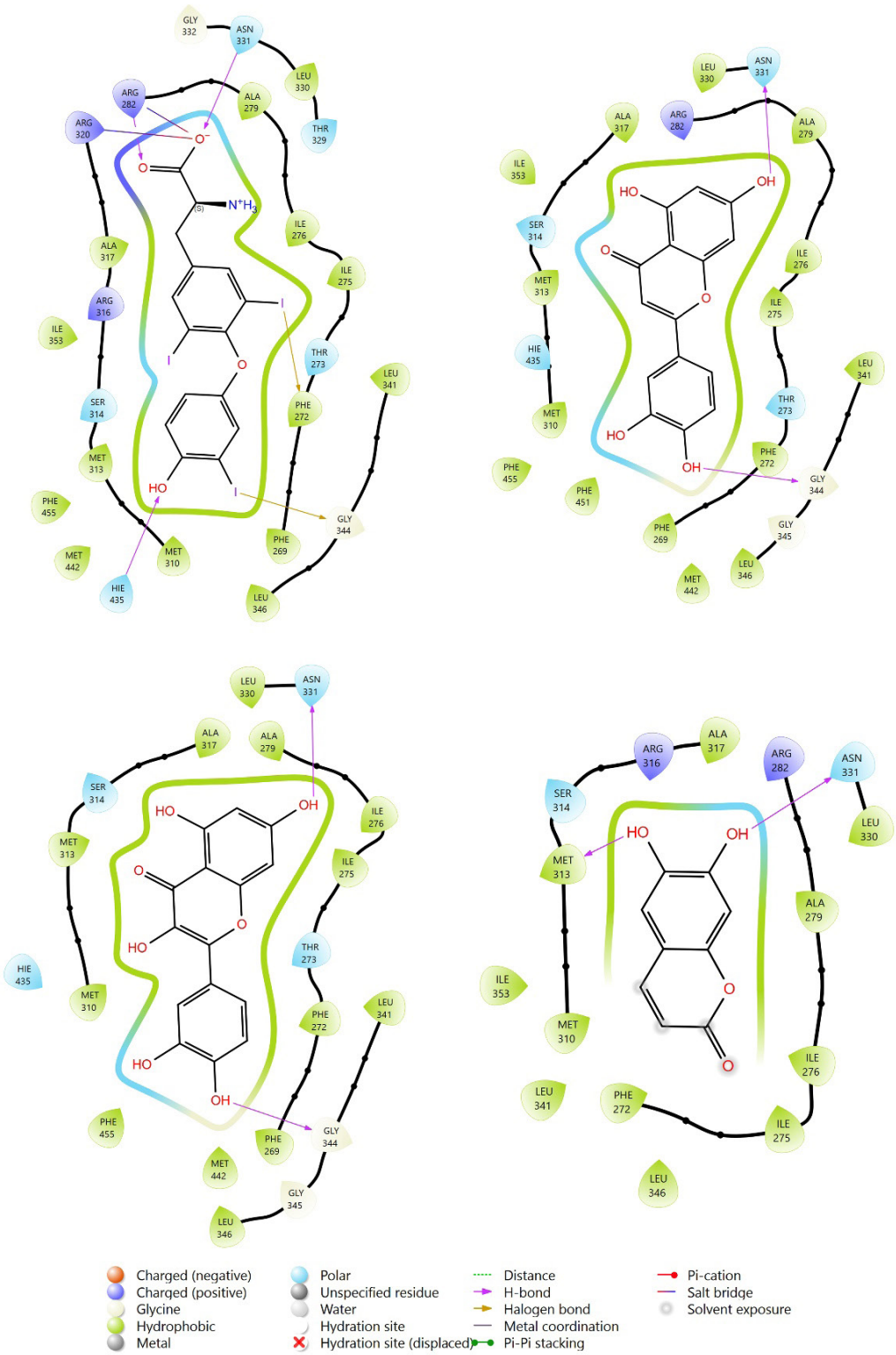

Figure 5: The 2D Ligand interaction diagram of the native ligand (top left), Luteolin (top right), Quercetin (bottom left) and Esculetin (bottom right).

Discussion

This manuscript examines the relationship between autoimmune thyroiditis and insulin resistance in the adult Turkish population. A retrospective analysis of clinical measurements from 34 participants reveals a significant prevalence of insulin resistance, with more than half exhibiting this condition, underscoring the importance of monitoring and managing it to prevent further complications (14). The study also highlights a higher prevalence of autoimmune thyroiditis in females, aligning with existing literature (5).

The research found a positive correlation between insulin resistance and blood glucose, insulin, HbA1c, and HbA1c (IFCC) levels, highlighting the need for enhanced blood glucose monitoring in individuals with autoimmune thyroiditis (15). No significant relationships were found between insulin resistance and hemoglobin, anti-TPO, anti-thyroglobulin, ferritin, vitamin B12, and vitamin D levels, indicating that insulin resistance may not impact these parameters in the context of autoimmune thyroiditis. This highlights the complexity of insulin resistance's role and the need for further research (16).

The study suggests a potential link between vitamin D levels, hemoglobin, anti-thyroglobulin, and vitamin B12 levels (17). This suggests a potential link between vitamin D status and certain health outcomes in autoimmune thyroiditis, but further research is needed to fully understand these associations.

G. aparine is traditionally used for natural therapy, particularly for hypothyroidism. Fourteen compounds were identified in its methanolic extract (11). To elucidate the molecular mechanism, we used in silico tools like molecular docking.

Prior to any computational modelling studies, a validation step is a must (18). In an attempt to validate our docking protocol, co-crystal ligand (T3) was docked into the binding site of Thyroid Hormone Receptor. The predicted binding geometry was then superimposed on the x-ray crystal structure. The room mean standard deviation (RMSD) was found as 0.34, which is an acceptable value. Then, we applied the same protocol (Glide SP) (19) to the docking of compounds reported in *G. aparine* methanolic extract. Amongst them, 12 compounds yielded successful poses. Table 4 shows Luteolin, Quercetin, and Esculetin have promising binding scores compared to the co-crystallized ligand.

Figure 5 demonstrates the binding contacts of all four molecules, including T3, with a hydrogen-bond connection at Asparagine 331 in all instances. This indicates that Asparagine 331 plays an essential role in the activity of the ligands against the Thyroid Hormone Receptor. Luteolin and Quercetin display interactions between hydrogen bonds at both ends, leading to enhanced stability and, due to their larger molecular size, yielding higher binding scores in compared to Esculetin.

The insights from this study have important clinical implications, potentially influencing management and treatment strategies for autoimmune thyroiditis. Further studies are needed to explore the relationships between autoimmune thyroiditis, insulin resistance, and other clinical parameters to improve patient care and outcomes (15).

Conclusion

This study highlights the significant interplay between autoimmune thyroiditis and insulin resistance in the Turkish adult population, with a high prevalence of insulin resistance. Key findings show a crucial link between insulin resistance and blood glucose and HbA1c levels, emphasizing the need for vigilant monitoring. No significant correlation was found between insulin resistance and hemoglobin or vitamin D levels. The study underscores the complexity of autoimmune thyroiditis and its metabolic implications, highlighting the need for a multifaceted management approach.

G. aparine, traditionally used for hypothyroidism, contains Luteolin, Quercetin, and Esculetin in its methanolic extract, which were predicted through molecular docking simulations to bind successfully to the Thyroid Hormone Receptor. These findings, along with the study's insights into the interplay between autoimmune thyroiditis and insulin resistance, emphasize the need for further research to explore underlying mechanisms and develop targeted treatments.

Declaration

Conflicts Of Interest /Competing Interests

The authors declare that they have no conflicts of interest.

Funding Statement

This study had no external funding.

Ethics Approval

This study was approved by the Ethical Committee of Biruni University, which granted approval under the protocol number 2022/75-12.

Authors' Contributions

ESA and SG conceptualized and designed the study. ESA organized the database, performed the statistical analysis, ESA and SG wrote the first draft of the manuscript. ESA edited the final version of paper. All authors approved the final version of the manuscript.

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